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Article

Risk factors associated with lentivirus seroprevalence in sheep and goat herds from northeastern Mexico

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Abstract:

A cross-sectional study was conducted with the purpose of determinate the risk factors associated with the serological frequency of small ruminant lentivirus (SRLV) in sheep and goats from northeastern Mexico. From 128 herds, 71 of goats, 32 of sheep and 25 mixed herds (goats + sheep), 768 individual sera were collected from animals ≥1 yr old. From each herd, 4 to 5 serum samples were mixed and analyzed by ELISA to identify antibodies against SRLV glycoprotein 135. Samples were obtained from randomly selected animals in 2019 and 2020. A questionnaire was applied to the producers and the data were analyzed to

determine the risk factors associated with herd seropositivity by logistic regression. The proportion of seropositive herds, overall, was estimated at 50.6 %. According to the type of herd, seropositivity in goat herds was 62.0 %, in sheep herds 25.4 % and 50.2 % in mixed herds. The risk factors associated with the presence of antibodies against SRLV were the presence of animals with arthritis, veterinary care, reuse of needles, nerve alterations, low pregnancy rate, type of herd and mastitis. Serological frequency indicates a high endemicity of SRLV in small ruminant herds from northeastern Mexico.

Key words: Retrovirus epidemiology, Small ruminants, Arthritis, Veterinary care, Biosecurity on farms.

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Introduction

In northeastern Mexico, sheep and goats are the two species of ruminants with the greatest territorial dispersion and form one of the main economic livelihoods for the rural population of this area[1,2]. In most of the herds from this area, a semi-extensive management system is practiced, in which the animals graze during the day, and before the spend the night in artisanal pens made of plant material from the region. Usually, no protein supplement, vitamins are offered, or adequate sanitary management is applied. Reports associated with health, reproduction and productivity disorders are common in herds[3,4,5]. Obviously, the lack of technical assistance, training, absence or lack of biosecurity, among others, contribute to these health problems[2,6].

Of the viral agents that affect sheep and goats, the infection caused by the small ruminant Lentivirus (SRLV) has become relevant in recent years[7,8]. SRLV is a non-zoonotic virus of the genus *Lentivirus*, subfamily *Orthoretrovirinae* and family *Retroviridae*, highly contagious and infectious among goats and sheep[9]. Initially, SRLV was named caprine arthritis encephalomyelitis virus or ovine progressive pneumonia virus (also called Maedi-Visna virus) since it was considered as two different pathogens specific to goats and sheep, respectively. However, it has been recognized that this virus can cross the species barrier and infect both ruminants[10,11]. In addition, molecular genetic studies have shown that, genetically, SRLV is the same virus, so it is currently recognized as a single virus with viral variants adapted to goats and sheep[12]. SRLV infections are lifelong and are characterized

by causing a chronic multisystem inflammatory disease, with slow and progressive development, that may or may not manifest itself clinically in the life of the animal[13]. They are characterized by gradual emaciation that leads to poor body condition and shortness of breath associated with interstitial pneumonia; alterations in the central nervous system, multiple arthritis and indurative mastitis in both species[9]. The clinical manifestation depends on the genetic characteristics of the infecting SRLV strain, its tissue tropism, the affected animal species and its genetics[9,14].

SRLV infections have a worldwide distribution[7] and are associated with significant economic losses[15,16]. In Mexico, the serological presence of SRLV in goats from Mexico was reported in 1985[17] and the isolation of the virus in 1999[18]. The serological presence of SRLV in goat herds from northeastern Mexico was reported in 1994[19]. Initially, a higher seroprevalence of SRLV was estimated in goat herds under intensive management in milk production and newly imported from the United States of America[17,19]. In Mexico, serological detection([20,21,22] and pathological damage associated with SRLV infection in goats and sheep[23] have been reported. Until 2012, Mexico was considered free of SRLV infection in sheep, but this infection is currently considered endemic and is within group 3 of diseases and pests in the national territory[24]. Serological and molecular evidence of SRLV infection in Mexican Pelibuey sheep was demonstrated in herds of Jalisco, Veracruz and Chiapas[25], State of Mexico and Querétaro[22]. However, studies of the presence, effects and impact of SRLVs on the health and productivity of goats and sheep from Mexico are scarce. The coexistence and multiple interrelationships between small ruminant populations in Mexico, particularly in the northeast of the country, usually increases the risk of acquisition and spread of SRLV and other pathogens[6,22]. It is known that sheep and goats can harbor multi-species infectious agents with the potential to affect these and other animal species and even humans[26,27]. In fact, SRLV can be considered within this category, so associated infections could trigger disease outbreaks and mortality. Given these conditions, a high serological frequency of SRLV at the herd level is considered and can be potentiated by at least one risk factor. Therefore, the objective of this study was to estimate the seroprevalence and determine the risk factors associated with Small Ruminant Lentivirus infection in sheep and goat herds in northeastern Mexico.

Material and methods

Location and characteristics of herds

A cross-sectional study was conducted, selecting 128 herds located in northeastern Mexico, in the states of Coahuila, Nuevo León and Tamaulipas. The management system of the herds was mostly semi-extensive. In general, the animals showed nutritional, reproductive and health complications.

Number of herds and animals sampled

A total of 768 animals were sampled from 71 goat herds, 32 sheep herds and 25 mixed herds (n=128 herds). For the state of Nuevo León, the sampling considered the total number of ranches registered in the 2017 list of beneficiaries of the Sustainable Livestock Production and Livestock and Beekeeping Management Program obtained by the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food of Mexico. For the states of Coahuila (Laguna Region) and Tamaulipas, the animals sampled were herds of farmers who, in a direct interview, expressed their desire to cooperate.

The sample size of 128 herds and 768 animals was calculated using the computer program EpiMuestra[28]. Because there was no information on SRLV infections for goats and sheep in northeastern Mexico, the following was considered: an expected prevalence of 50 %, a confidence level of 95 % and absolute accuracy of 5 %. The sampling was in two stages, first selecting the herds and then the animals within each herd, arbitrarily considering a design effect of 2[28]. The sampling unit for analysis was the herd. Serological analyses were analyzed by groups that corresponded to a homogeneous mixture of 4 to 5 sera per herd[29] at a rate of 200 µL per animal. The herd whose serum mixture was positive in the commercial ELISA test was considered positive.

Serum samples and their handling

Serum samples were obtained between the autumn of 2019 and late spring of 2020. Blood was obtained by puncture of the jugular vein and vacuum tubes with coagulation activator gel (Becton Dickinson, www.bd.com). The samples were identified and transported to the

laboratory under refrigeration conditions at 7 °C (±3) in a polystyrene container. In the laboratory, the sera were separated from the clot after centrifuging the tubes at 2,500 rpm for 5 min. Each serum sample was deposited in new sterile plastic tubes of 2 ml. Each tube was labeled with its individual code, date and origin. All samples were stored at -20 °C in the serum bank of the Laboratory of Virology of FMVZ-UANL, until their use in the ELISA test.

Field information collection

The identification of possible risk factors was determinated based on the responses of the farmers in an individually applied survey. The survey consisted of 30 questions, and they included aspects of the type of farm, health and animal health aspects, as well as the identification and location of the herd.

Detection of anti-SRLV antibodies

The detection of anti-SRLV antibodies was performed by competitive ELISA with the commercial kit Small Ruminant Lentivirus Antibody Test Kit, cELISA, (WMRD Inc., Pullman, WA, USA). This test detects antibodies directed against highly conserved antigenic sites of glycoprotein 135 of caprine arthritis encephalomyelitis virus. The sensitivity and specificity reported for the test was 100 % and 96.4 %, respectively[30]. The reading of the reaction in each well was made at an optical density of 650 nm in the ELISA reader (ELx800, Bio-Tek®) and using the computer package KC Junior software (www.biotek.com).

The presence of antibodies was derived from the calculation of the percentage of inhibition according to what was recommended by the manufacturer, using the formula:

$I = 100 \{1 – (\text{OD of the sample}/ \text{OD of the NC})\}$

Where: I is the percentage of inhibition; OD is the optical density detected; NC is the negative control.

For the validation of the test, an average of the OD of the NC ≥ 0.300 was considered. If the *I* value of the sample was ≥ 35 %, it was considered as positive, while an I <35 % as negative[30].

Statistical analysis

From the positive reactions in the ELISA test of each herd, the actual seroprevalence of SRLV was estimated by means of the online tool WinEpi version 2.0[31]. For the estimation of proportions and 95 % confidence intervals ($CI_{95\%}$), the sensitivity and specificity reported by the manufacturer of the ELISA kit was included[30]. To determine the association between risk factors and SRLV seropositivity, initially, possible risk factors were identified in a univariate analysis by the Chi-square test or Fisher's exact test (PROC FREQ). Those factors with a (P>0.20) were subjected to a multivariate logistic regression analysis (Table 1) by means of the LOGISTIC procedure. Factors significant to Fisher's exact test with fewer than 5 observations were not included in the logistic regression analysis. All analyses were performed using the statistical package SAS of 2010.

Results and discussion

Herd seroprevalence

The herd seroprevalence value against SRLV of 50.6 %, obtained in the present study (Table 1), is consistent with those obtained in other parts of the world[30,31,32] but contrasts with previous studies conducted in Mexico[21,33,34,35]. Recently, Martínez-Herrera *et al*[33], using an indirect ELISA test, reported a lower herd-level seroprevalence in Creole goats from Veracruz, Mexico, with 6.4 %. Also, Torres-Acosta *et al*[21], using agar gel immunodiffusion (AGID), reported in 2003 an apparent seroprevalence of 3.6 % in goat herds, mostly Creole from the state of Yucatan, Mexico. Previously, in 1984 Adams *et al*[34], using AGID, reported at the individual level in goats from the State of Mexico and Guanajuato serological frequencies of 22.1 % and 6.3 %, respectively. These same researchers mentioned not finding antibodies in native Creole goat herds[34]. Santiago *et al*[35], using the same ELISA test as the present study, found a herd-level seropositivity of 41.3 % in samples of goats from the state of Guanajuato, Mexico. According to the above, the design of the study, the management, type and purpose of the herds, as well as the lack of biosecurity measures against SRLV probably influenced the seropositivity parameters of the present and each of the previous studies. In 1984 and 1985, a high seropositivity in goats of dairy breeds imported into Mexico and absence of seropositivity in native Creole goats were reported[17,34]. These observations and data from the present study suggest that SRLV entered native Creole herds from northeastern Mexico perhaps through contact with imported breed goats for the purpose of improving productivity. In the present study, no significant difference was found[36] between

the seroprevalence of the types of herds, of goats (63.0 %), of sheep (25.4 %) or mixed (50.2 %). These data contrast with those obtained in similar herds from other countries[30,31,32] in which the seropositivity indices are relatively low compared to those of the present study. However, this coincides with what has been reported in previous studies for the management of a single species, either sheep or goats[32,36,37] and when they are managed under mixed conditions[36]. The type of serological test used, the management and characteristics of the environment could explain the differences found.

Table 1: Prevalence of antibodies against small ruminant lentivirus (SRLV) in sheep and goat herds in northeastern Mexico

Herd	n	(+)	ActP	$CI_{95\%}$
Goats	71	45	62.0	50.7 – 73.3
Sheep	32	9	25.4	10.3 – 40.5
Mixed (goats + sheep)	25	13	50.2	30.6 – 69.8
Overall	128	67	50.6	41.9 – 59.2

ActP = actual prevalence, *Sensitivity (100%) and specificity (96.4%) of the ELISA test[30], 95% confidence level[31].

Risk factors associated with serology at the herd level

After analysis in contingency tables, a total of 21 factors out of 30 were selected to evaluate their association with SRLV seropositivity in goat and sheep herds. The risk factors that contributed significantly to the explanation of SRLV seropositivity were: type of herd, veterinary care, multiple use of needles, low pregnancy rate, presence of animals with arthritis, report of nerve alterations and animals with mastitis; these last three alterations associated with chronic inflammatory processes (Table 2). The logistic regression analysis showed a significant effect of the same factors as in Fisher's exact test or Ji-square test; but the following factors were not included: herd size, introduction of animals, quarantine, biosecurity and mixed herds because each of them had ≤5 observations. Several reports[32,33,36] have indicated these last variables as risk factors so they were included in the discussion. Table 2 shows the risk factors associated with SRLV seropositivity in goat and sheep herds from northeastern Mexico. In northeastern Mexico, it is relatively common to find mixed goat-sheep herds. The coexistence of both species could facilitate the transmission of SRLV not only through direct contact but also through the intake of colostrum or milk[37,38] and through other management practices such as the use of needles in several animals during the application of medicines, vaccines or identification earrings[6,37,39].

Table 2: Herd seroprevalence and risk factors for SRLV seropositivity in goat and sheep herds from northeastern Mexico

Variable		n	Seroprevalence	*P*	Odds Ratio ($IC_{95\%}$)
ART	Yes	63	82.5	<0.0001	31.3 (6.7-142.8)
	No	65	23.1		1
CARE	Yes	19	94.7	<0.0001	11.9 (1.0-142.9)
	No	109	44.9		1
NEED	Yes	69	63.8	0.005	9.6 (2.1-43.5)
	No	59	38.9		1
NERV	Yes	47	78.7	<0.001	7.4 (1.9-28.6)
	No	81	37.0		1
PREG	Yes	50	74.0	<0.0001	6.8 (1.8-25.6)
	No	78	38.5		1
HER	Caprino	71	63.4	0.0041	5.4 (1.0-28.2)
	Mixto	25	52.0		2.2 (0.4-12.3)
	Ovino	32	28.1		1
MAST	Yes	66	71.2	<0.0001	4.9 (1.3-17.9)
	No	62	32.5		1

CI = class intervals, ART = presence of arthritis, CARE = veterinary care, NEED = repeated use of needles, NERV = presence of nerve alterations, PREG = low pregnancy rate, HER = type of herd, MAST = presence of mastitis.

A strong association was found between the type of herd, of goats (OR 5.4; $CI_{95\%}$= 1.0-28.2) or mixed (goats + sheep) (OR 2.1; $CI_{95\%}$= 0.3-12.3), with SRLV seropositivity. Similar observations have reported that the presence of goats is a risk factor that contributes to the SRLV seropositivity in sheep[39,40]. Herd size has been reported as another important factor that influences SRLV seropositivity, because one of the routes of transmission of this virus is through direct contact between infected animals[36,39,40]. However, this variable was excluded from the logistic regression analysis due to the low number of observations and to meet the data quality criteria for analysis. In the present work, a strong association was found between the presence of animals with arthritis (OR 31.2; $CI_{95\%}$= 6.7-142.8) and with mastitis (OR 4.8; $CI_{95\%}$= 1.3-17.8) in seropositive herds. SRLV infections are characterized by being strongly related to these clinical-pathological conditions[40,41,42]. For sheep, a high association has been reported between the occurrence of mastitis and SRLV infection in endemic

herds[9,39,40]. In a recent study, it was determined that when the goat farmer recognizes the presence of arthritis, SRLV infection is widespread in the herd[42]. It has also been proposed that the development of arthritis depends on the genetic characteristics of the infecting SRLV strain[43]. What was observed in the present study indicates that animals with chronic arthritis are present with high frequency in seropositive herds regardless of the animal species and the type of management.

An association was found between reproductive problems and SRLV seropositivity. Herds in which the producer recorded a low pregnancy rate (≤50 %) were more likely to be seropositive than those with pregnancy rates ≥50 %. Few studies have focused on knowing the impact of SRLV on reproduction in small ruminants. Recently, the ability of this virus to induce intrauterine infections in small ruminants and be transmitted via semen either with artificial insemination or natural mounting was reported[44]. However, no association was found between the presence of abortions or animals with low birth weight in the herd, which contrasts with previous studies in which the delayed development of newborn kids was associated with the seropositivity of the mother[37,39]. Probably, the differences between the two observations are explained due to the nature of both studies or the bias in the responses given by the producers in the present research. Interestingly, an association (P<0.0001) was found between SRLV seropositivity and veterinary care (OR=11.9; $CI_{95\%}$= 1.0-142.8). An important form in the horizontal transmission of SRLV is contact with humans, particularly the movement of veterinarians and workers between and within herds[39]. It is possible to consider that veterinary care would play an important role in controlling SRLV infection; the increase in seropositivity in herds of small ruminants with vectorization by the veterinarian from one herd to another during their visits has been reported[39]. The data obtained in the present work probably reflect that the same veterinarian cares for different herds. This was not included in the surveys, allowing the horizontal transfer of the virus in the region studied due to ignorance of the presence of the virus in the region, its consequences and forms of dispersion. One study indicates that the presence of humans, as well as the number of employees and years of experience in management within herds are related to the presence and circulation of the virus[39].

On the other hand, the absence of biosecurity, hygiene and disinfection measures greatly increases the presence of SRLV infection[42]. Therefore, it is necessary to make producers aware of this agent, as well as to let them know the biosecurity guidelines to prevent the circulation of the virus in their herds. Based on the above, the epidemiological observations indicated for more than 25 yr for SRLV in goats from Mexico are confirmed and expanded[17,19,34]. In addition to this, the present work is the first serological report of SRLV infection in sheep from northeastern Mexico.

SRLV is considered as a single virus with genetic variants adapted to goats or sheep[8,9]. The possibility of cross-infections[10,11] and the isolation of recombinants among the genetic

variants of the virus have also been reported[41]. Given the above, it is interesting to determine if the serological response found in small ruminants is directed towards genetic variants of SRLV adapted to goats or sheep[22]. Likewise, to be able to specify if recombinant SRLVLs[8,10] that have managed to adapt to both sheep and goats in the area[11] circulate in the northeast of Mexico.

Conclusions and implications

SRLV seropositivity in sheep and goats from northeastern Mexico is relatively high. This is the first serological report of SRLV infection in sheep from northeastern Mexico. The estimated seroprevalence and risk factors detected in seropositive herds should be considered in the design of biosecurity programs and public policy applied to the health and productivity of goat and sheep herds in Mexico.

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